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Insertion Sites of Feline Leukemia Virus

Alexa M. Salsbury

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Insertion Sites of Feline Leukemia Virus

Abstract
Feline Leukemia Virus (FeLV) is a transmittable RNA retrovirus that inhibits feline immune systems and predisposes its host to infections and diseases. Much is unknown about FeLV, including the nature and location of its insertion sites. Identifying insertion sites of the provirus can provide information about mechanisms of tumorigenesis and identify new protooncogenes. To search for insertion sites, gene-specific primers were designed and a genome walking method was optimized for our application. Using the optimized process, blood and tissue samples were examined for FeLV provirus. The cat from which we received samples showed neurological symptoms before death; therefore, we hypothesized FeLV sequences would be present in brain tissue. We also hypothesized that brain sequences and insertion sites would differ from specific sequences found in blood and other tissue samples. Exogenous FeLV-B sequences were successfully amplified from blood, brain and lymph tissues and no differences were found between sequences. Exogenous sequences in the brain indicate the presence of cellular receptors recognizable by FeLV-B. This is significant because previous research suggested that brain cells have a resistance to the virus. Furthering knowledge of mechanisms and locations of insertion can lead to a more complete understanding of FeLV disease progression, ultimately leading to the development of more effective treatments, conserving the health of domesticated and large endangered feline species.

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retrovirus, FeLV-B, cellular receptors, touchdown PCR, FeLV proviral insert

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Abstract

Feline Leukemia Virus (FeLV) is a transmittable RNA retrovirus that inhibits feline immune systems and predisposes its host to infections and diseases. Much is unknown about FeLV, including the nature and location of its insertion sites. Identifying insertion sites of the provirus can provide information about mechanisms of tumorigenesis and identify new proto-oncogenes. To search for insertion sites, gene-specific primers were designed and a genome walking method was optimized for our application. Using the optimized process, blood and tissue samples were examined for FeLV provirus. The cat from which we received samples showed neurological symptoms before death; therefore, we hypothesized FeLV sequences would be present in brain tissue. We also hypothesized that brain sequences and insertion sites would differ from specific sequences found in blood and other tissue samples. Exogenous FeLV-B sequences were successfully amplified from blood, brain and lymph tissues and no differences were found between sequences. Exogenous sequences in the brain indicate the presence of cellular receptors recognizable by FeLV-B. This is significant because previous research suggested that brain cells have a resistance to the virus. Furthering knowledge of mechanisms and locations of insertion can lead to a more complete understanding of FeLV disease progression, ultimately leading to the development of more effective treatments, conserving the health of domesticated and large endangered feline species.
Acknowledgements

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Introduction

Overview

Feline Leukemia (FeLV) is a gamma retrovirus that integrates into host DNA, where the virus remains for the lifespan of the host. The virus inhibits the immune system of various feline species and predisposes its hosts to infections and diseases. FeLV is particularly threatening because of its symptoms and contagious nature. Transmitting the virus can occur through contact with bodily fluids of an infected cat and therefore FeLV is a leading killer among domesticated and wild cats. Reported recently, FeLV has infected large cat species such as the *Puma concolor* and the *Lynx pardinus*, posing a risk to endangered species and demonstrating the possible vulnerability of all felines to the virus.

Feline Leukemia Virus is separated into four subgroups (A, B, C, and T), each of which uses different cellular receptors to enter the host and bring rise to different symptoms. All infected felines carry FeLV-A, a horizontally transmissible and weakly pathogenic subgroup. FeLV-B and FeLV–C subgroups are only present when FeLV-A is present. FeLV-B occurs in around half of infected cats, and is most often diagnosed in hosts that have developed tumors. FeLV-C is less common, occurring in approximately 5% of FeLV-positive cats, and is associated with acute anemia. FeLV-T is the most rare subgroup, accounting for <1% FeLV cases. It has been linked with immune suppression similar to feline immunodeficiency virus. Currently, there are no effective treatments for any subtype of FeLV.

Mechanisms of pathogenesis are not fully understood; however, studies of FeLV-host interactions have been used to develop theories for FeLV insertion mechanisms. Current
theory suggests that FeLV inserts randomly into the feline DNA. However, studies of FeLV-induced tumors have identified sites into which FeLV has inserted more often than would be predicted statistically, including proto-oncogenes –c-myc, pim-1 and flvi-2. Our research has focused on developing a reliable and repeatable method to search for integration sites and gaining more information about the host genetic sequences into which each strain of FeLV integrates.

**FeLV Structure**

The Feline Leukemia virus is a spherical gammaretrovirus with an envelope and a reported diameter of approximately 110 millimicrons. The structure and viral components, including surface proteins, integrase, matrix, capsid, viral RNA genome, and reverse transcriptase, of FeLV are depicted in Figure 1. The membrane surrounds the capsid and has protruding glycoproteins, biologically active dimers. Glycoproteins are surface proteins that facilitate entry into a host cell because they have transmembrane subunits that bind to a receptor and C-terminal region, allowing fusion between the virus and cell membrane. Under the surface proteins is a matrix that houses a capsid. The capsid is a protein shell that surrounds its genetic material. Enclosed within the capsid are viral enzymes, viral proteases, reverse transcriptase, and integration proteins. Integrase is a specific viral enzyme that is produced by retroviruses and enables genetic material to be integrated into host DNA.
Figure 1: Structure of FeLV. This illustration contains viral components described above as well as a single stranded RNA depicting the viral RNA Genome.

After the virus enters the cell, viral reverse transcriptase make DNA copies of the RNA genome. In the nucleus, integrase inserts proviral DNA into the host genomic DNA where it can then be transcribed, producing mRNA. mRNA allows the translation of viral proteins and RNA for new virions. Budding and cleavage then occurs in the cell membrane.  

The FeLV genome is made up of three genes- the gag gene, pol gene, and env gene- which code for nine viral proteins. The gag gene codes for four internal structural proteins that connect the retrovirus to the envelope, form primary structural proteins for the virus’ core, and form nucleocapsid proteins. The pol gene codes for three viral enzyme proteins that allow synthesis of DNA from single stranded RNA templates and integration of viral DNA provirus into the host genome. Lastly, the env gene codes for two glycoproteins that identify
the viral subgroup. The proviral DNA is illustrated below:

**PROVIRAL DNA**

![Diagram of proviral DNA]

Figure 2: Proviral DNA. The structure of the integrated FeLV provirus, including the viral genes (gag, pol, env) and the long terminal repeat (LTR). Natural isolates of FeLV show great variation in the LTR, often with a duplicate enhancer or other repeat sequences. Specific LTR variants have been linked to different disease progression and outcome, though the mechanisms are not fully understood.

**Prevalence in Nature**

FeLV is an increasing issue in nature because the virus is highly contagious. Transmission occurs through contact with bodily fluids of an infected cat including saliva, mucus, urine, feces, milk, and blood. Merely a scratch or a shared litter box can facilitate the transmission of the virus. Viremia often sets in very quickly. The short time period between infection and onset of symptoms makes containment of the virus very difficult. Infection can also result in the host having a latent period where it is infected but does not experience symptoms. A cat can test negative during a latent period and therefore would not be perceived as a threat. In this situation, the infected cat is often integrated into a home. This leads to transmission because the feline still has potential to spread the virus after the latent period. For this reason, many veterinarians suggest that a cat be tested twice for FeLV, the
first test should be administered immediately and the second test should be used to verify sixty days later. It is important to note that very rarely infected felines can clear the virus to an undetectable point and therefore never transmit the virus. In this case, the cats are not viremic, but do have proviral DNA. Still, research shows that these cats are more susceptible to illness for unknown reasons.\textsuperscript{[11]}

FeLV is a virus that has been found in felines all around the world. Prevalence of the infection varies depending on environment, access to vaccinations, age, and lifestyle. While approximately 3% of cats in the United States have been found infected, the amount of infected kittens is more than 13%, and the amount of infected wild cats is thought to be over 40%. Not all cats are tested so estimating the statistical prevalence of FeLV is challenging. Researchers estimate that as much as 15% of the feline population carries FeLV. Still the most grisly fact is that FeLV is always fatal.\textsuperscript{[12]}

A large concern in the field of research for FeLV came when cases of infection were found in the Florida panther, one of the most endangered feline and animal on earth. Researchers do not know how the disease was introduced to the large, wild feline species; however, preservation efforts were strong and vaccines were administered to as many Florida panthers as possible.\textsuperscript{[3]} This outbreak demonstrates the potential danger of the disease to all feline species and should bring about urgency in research.

FeLV Subtypes

Feline Leukemia Virus is separated into four subtypes- FeLV-A, FeLV-B, FeLV-C, and FeLV-T. Each subtype uses different cellular receptors to enter the host and bring rise to different symptoms summarized in Table 1. All felines infected with the virus carry subtype
A. Both FeLV-B and FeLV-C are thought to arise from recombination of FeLV-A with endogenous viral sequences and mutation. The four subtypes have been shown to have differing glycoprotein sequences in the envelope, resulting in different pathogenesis.\[^{13}\] For example, FeLV-T is the only subtype to require the virus to bind to the receptor as a prerequisite for transactivation. Of the four subtypes, FeLV-A is the only one horizontally transmitted.

It is important to note that a small percent of infected felines develop a non-productive infection and carry the integrated provirus without the presence of the virus in the blood. However, they are still infected, and viral production can activate in times of duress.\[^{14}\]

**Table 1: FeLV Subtypes.** The symptoms, origin, and characteristics of FeLV subtypes are compared to clarify differences between subtypes and to enhance understanding.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Symptoms</th>
<th>Origin</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>FeLV-A</em></td>
<td>Hematopoietic neoplasia,</td>
<td>Original FeLV strand; all infected cats carry</td>
<td>- Pathogenic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Causes structural changes in host</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Highly contagious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Present in all infected feline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Affects tissues in the liver, kidney, small intestines, and T cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Uses thiamine transport protein receptor for entry</td>
</tr>
<tr>
<td><em>FeLV-B</em></td>
<td>Typically asymptomatic</td>
<td>Polytropic virus found in conjunction with FeLV-A; arises by recombination of FeLV-A with endogenous retroviral sequences</td>
<td>- Non-pathogenic by itself</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Pathogenic when recombined with FeLV-A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Uses Pit1 and Pit2 receptors for cell entry</td>
</tr>
<tr>
<td><em>FeLV-C</em></td>
<td>Anemia, erythremic myelosis (leukemia)</td>
<td>Polytropic virus found in conjunction with FeLV-A; arises by mutation of env genes from FeLV-A subgroup</td>
<td>- Does not replicate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Non-contagious</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Affects stem cells that give rise to red blood cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Uses heme exporting protein receptor</td>
</tr>
<tr>
<td><em>FeLV-T</em></td>
<td>Lymphopenia, neutropenia, fever, diarrhea</td>
<td>Evolved from mutation and recombination of FeLV-A</td>
<td>- Only subtype to require the virus to bind to the receptor as a prerequisite for transactivation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Affects T-cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Uses Pit1 and co-receptor feLIX for cell entry</td>
</tr>
</tbody>
</table>
Genome Walking with Polymerase Chain Reaction

Genome walking is a biological method used to determine unknown DNA sequences that lie adjacent to known sequences. This efficient and reliable method uses Polymerase Chain Reaction (PCR). PCR is an effective biological technique for selectively amplifying specific DNA segments, resulting in anywhere from thousands to millions of copies of that DNA sequence. The technique has a few components including a DNA template, DNA polymerase, primers, and nucleotides. The DNA template is the piece of DNA that contains the target sequence. DNA polymerase is an enzyme that synthesizes new strands of DNA complementary to the target sequence. Lastly, primers are short, single-stranded DNA pieces that are complementary to the target sequence and allow polymerase to begin synthesis.

PCR begins by heating a sample to denature DNA. The DNA is separated to two single-stranded pieces of DNA. DNA polymerase then uses these original strands as templates to synthesize two new stands of DNA. The two newly synthesized copies are then used to create two new copies. This cycle, of denaturing and duplicating, repeats itself resulting in numerous copies of the sample DNA. These PCR processes are automated and take place in a thermocycler that can be programmed to varying conditions. Figure 3 gives you a visual illustration of PCR amplification:
Figure 3: PCR Amplification. (a) This section depicts the first PCR cycle. The double-stranded DNA template is illustrated with primers represented by the blue and red pieces. Primers anneal, the original DNA strand denatures, and new strands are synthesized. Green segments represent new strands of DNA. (b) This section depicts the second PCR cycle in which the process shown in section (a) is repeated. (c) This section shows the accumulation of the target product. Two copies of the template DNA are produced per cycle, leading to exponential growth.

Touchdown PCR (TD-PCR) is a variation of PCR that uses a higher annealing temperature, the upper limit of the primer’s melting point. This results in specific base paring of primer to template. It is used to increase specificity of amplification by avoiding nonspecific primer binding in the first few cycles with high annealing temperatures. Temperature is gradually lowered throughout the cycling process. Nonspecific primer
binding is an issue because unwanted sequences can interfere with efficient amplification of sequences of interest. By lowering the annealing temperature of the cycling process progressively, the target sequence is selectively amplified at the beginning, avoiding significant amplification of unwanted sequences.

**Materials and Methods**

**DNA**

Blood and various tissue samples were donated by a local veterinarian who works for a FeLV shelter, Leuk's Landing Leukemia Rescue for FeLV-positive cat samples. Blood samples from two cats, labeled cat 8 and 23, were used. Tissue samples included brain, liver, spleen, kidney, and mesentery lymph samples. These samples were provided from cat 23.

**Primers**

Primers for PCR amplification were designed using the National Center for Biotechnology Information's Primer-BLAST tool. The primers were designed to be specific for the template of interest and were cross checked using the nucleotide database. The first set of primers used to confirm the presence of FeLV-B was RB53 and RB17. RB53 is a forward primer and RB17 is a reverse primer:

- RB53 (F) ACAACGGGAGCTAGTG
- RB17 (R) TAGTGATATTGGTTCTTCG
Since optimal annealing temperature for Touchdown-PCR is based on melting temperatures of the primer-template pair and Gibbs free energy of oligonucleotides effects optimization of conditions, Table 2 helps understand the optimization of TD-PCR conditions.

**Table 2: Melting temperatures and maximum ΔG.** The table lists melting temperatures and maximum ΔG values for each primer used. Calculations were performed using IDT OligoCalc, with 0.25 μM each primer, 50 mM Na⁺, and 1 mM dNTPs.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Tm (°C)</th>
<th>ΔG (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BWF: CTAGTGAGGGGCGCGGATGCACCC</td>
<td>68.8</td>
<td>-55.55</td>
</tr>
<tr>
<td>BWR: CCGGACCTGGCAGTTTGTCAAGGTGT</td>
<td>66.8</td>
<td>-54.46</td>
</tr>
<tr>
<td>B1F: CCCCCATCCTGCTATCTATTCGCA</td>
<td>65.8</td>
<td>-55.05</td>
</tr>
<tr>
<td>B2F: GCCTTAAATGCCACCAGCCCAACAA</td>
<td>65.9</td>
<td>-55.48</td>
</tr>
<tr>
<td>B3F: TCCCCCATCCTGCTATCTATTCGCA</td>
<td>65.6</td>
<td>-54.67</td>
</tr>
<tr>
<td>B5F: TCCCGAGTAACACCTCACCATTCCA</td>
<td>65.3</td>
<td>-51.03</td>
</tr>
<tr>
<td>B1R: GGTCCAATTGAGCCACCGCCATGGAA</td>
<td>65.9</td>
<td>-54.74</td>
</tr>
<tr>
<td>B2R: TCGGGAATAGATAGGAGCAGGATGGGGG</td>
<td>65.8</td>
<td>-55.05</td>
</tr>
<tr>
<td>B3R: CGGCTAGATAGTGCACCCTCTGTATGT</td>
<td>65.8</td>
<td>-52.61</td>
</tr>
<tr>
<td>B5R: AGCCTGGGGTGCTTAAGGAACAGTCC</td>
<td>65.7</td>
<td>-50.35</td>
</tr>
</tbody>
</table>

The primers used can be visually represented on the proviral envelope shown in Figure 4:
Figure 4: Primer Information. Displays the four forward (blue) and four reverse (red) primer positions and orientations between 13-1411 base pairs (on the template). These primers were designed with high melting for use with a touchdown PCR protocol to help improve amplification of FeLV sequences.

DNA Extraction

Blood Samples

Extraction of DNA from blood samples was performed using PureLink® Viral RNA/DNA Purification Kit from Life Technologies. Life Technologies’ protocol was followed and the samples were stored at -80°C.

Tissue Samples

Extraction of DNA from tissue samples was performed using a Nucleospin Tissue Genomic DNA Extraction Kit from Clontech. Clontech’s protocol was followed and samples were stored at -80°C.

DNA Digestion

The use of restriction enzymes is very important to the DNA digestion process. The restriction enzymes used were DraI, EcoRV, PvuII, and Stul and are detailed in Figure 5.
Figure 5: Restriction Enzyme. Displays restriction enzyme cuts as predicted on two different FeLV templates (Accession Numbers NC_001940.1 and AB060732.1). The red portions stretch from approximately 6500 bp to 7300 bp and represent slight differences in the templates that could cause changes in the predicted digest.

DNA templates were digested with restriction enzymes Dral, EcoRV, PvuII, and Stul according to the Clontech Genome Walker manual. After the protocol was complete, reactions were incubated overnight at 37 °C.

Purification of Digested DNA

A Clontech NucleoSpin® Gel and PCR Clean-up Kit was used to purify the digested DNA samples according to the provided manuals. Once clean-up was complete, purified DNA samples were placed into new microcentrifuge tubes and stored at -20 °C.
Ligation of DNA to Adapters

A Clontech Genome Walker kit was used to ligate adapters to purified DNA digests. 4.8 µL of purified DNA samples were ligated following manufacture reagents and instructions. Samples were then stored at -20 °C.

Polymerase Chain Reactions

Primary PCR wells for a reaction were prepared with DNA from ligated samples and components from Clontech Advantage 2 PCR Enzyme System according to the user manual. If bands were present in the gel electrophoresis image of primary PCR products, a secondary PCR reaction was employed. Secondary PCR wells for a reaction were prepared with samples from the primary PCR and components from Clontech Advantage 2 PCR Enzyme System according to the user manual. Reactions were run under the same thermocycling conditions.

Initial conditions for PCR involved four steps. The first step was one cycle at 95°C for 10 minutes. The second step was forty cycles of 95°C for 30 seconds, followed by 60°C for 30 seconds, and 72°C for 80 seconds. Step three was one cycle at 72°C for 7 minutes and step four was one cycle at 40°C for 10 minutes.

Upon optimization of technique for our samples, Touch Down-PCR conditions were employed. This involved five steps. The first step was one cycle at 95°C for 60 seconds. The second step was seven cycles of 95°C for 30 seconds followed by 72°C for 3 minutes. The third step was thirty-two cycles of 95°C for 30 seconds followed by 67°C for 3 minutes. Step four had one cycle at 67°C for 7 minutes. The final step was a 4°C hold until removed and stored at -80°C. The conditions of the TD-PCR are depicted in Figure 6.
**Figure 6: PCR Cycling Conditions.** The Touchdown PCR program consists of an initial “hot start” at 95°C to activate the taq polymerase, 7 cycles with an annealing temperature of 72°C, 32 cycles with an annealing temperature of 67°C, and a final extension at 67°C. The high initial annealing temperature allows more selective binding of the FeLV primers to the genomic DNA, minimizing mispriming (non-specific annealing to the template).

**Gel Electrophoresis**

Gel Electrophoresis was performed using a 1.2% agarose gel. About 3 mL of Gel Red intercalating nucleic acid stain was inserted in the dissolved gel mixture before setting in the gel tray. Gels were run at 90 mV for approximately an hour. Time varied depending on the degree of separation of the bands. Gels were read on a Bio-Rad gel imaging system.

**DNA Extraction from Gels**

Bands of interest were directly extracted from secondary PCR gels. A UV lamp was used to make bands visible so that they could be cut out. Once bands were cut out from a gel they were weighed and placed in a column. 200 µL of buffer NTI was added to the column for each 100 mg of gel. NucleoSpin Gel extraction instructions were followed to clean up the
samples. Once clean-up was complete, concentrations of extractions were measured using Nanodrop instrument.

**Cloning and Transformation**

A PureLink® Quick Plasmid Miniprep Kit was used to isolate plasmid DNA and plasmid DNA samples were stored at \(-20^\circ\text{C}\). LB agar plates containing ampicillin (100 microliters/mL) or kanamycin (50 microliters/mL) were prepped. For cloning a TOPO TA cloning kit for sequencing from Life Technologies was used. 4 µL plasmid DNA samples, 1 µL of salt solution, and 1 µL of TOPO vector were mixed and incubated at room temperature. Previously prepared DH5 α-competent cells were used for transformation.

**Extraction of Colonies**

Colonies were extracted using PureLink Quick Plasmid DNA Miniprep kits and following manufacturer instructions. Concentrations of extractions were then measured using Nanodrop instrument and were prepared for sequencing.

**Sequencing**

Samples were prepared for sequencing by diluting to 50 ng/µL. Samples with measured concentrations lower than 50 ng/µL were sent to sequencing without dilution. Samples were submitted for standard Sanger sequencing at the University of Michigan Core Sequencing Lab.
Results and Discussion

The presence of FeLV-B was confirmed following PCR using primers RB53 and RB17. The PCR reactions were performed under the initial conditions, not TD-PCR. This yielded an amplicon of 857 bp. Figure 7 shows the agarose gel image obtained.

Figure 7: PCR Gel Electrophoresis Image. A 1.2% agarose gel image confirmed the presence of FeLV-B in genomic DNA extracted from blood samples of FeLV-positive cats.

These samples were extracted from the gel, cleaned up, and sent to the University of Michigan Core DNA Sequencing Lab for sequencing. A sequencing chromatogram was obtained for the amplicon from cat 10 and was confirmed to match FeLV-B proviral sequences. Figure 8 shows this chromatogram.
Figure 8: Sequencing Chromatogram. This is the sequencing chromatogram for the amplicon of FeLV-B obtained from Cat 10 blood extractions.

The chromatogram showed appropriate signal to noise ratio, little interference, and alignment with envelope regions. While alignment with envelope regions was confirmed some differences were observed. These differences are highlighted in Figure 9.
**Figure 9: Sequencing Chromatogram Differences.** This again shows the sequencing results for the amplicon of FeLV-B for Cat 10 (Figure 7). The sequence is shown aligned with the envelope region of FeLV-B (NCBI Accession Number NC_001940.1). Differences are highlighted in the sequence, and the corresponding sections from the chromatogram are shown. The differences are thought to be mutations.

Sequencing results shown in Figure 7, Figure 8, and Figure 9 display successful amplification of exogenous FeLV sequences from non-infected feline blood. These results also served as a template to design FeLV-specific primers for TD-PCR and genome walking. PCR amplification was then performed on tissue samples using the amended touchdown-PCR conditions. The gel imaging of primary and secondary PCR reactions using DNA extractions from FeLV infected brain samples is shown in Figure 10.
Figure 10: Primary and Secondary PCR from Brain Samples. An example of multiple bands obtained from Primary PCR and then Secondary PCR of brain samples with restriction enzymes StuI, DraI, PvuII-HF, and EcoRV-HF and using primer AP1- B1R.

These bands as well as bands from lymph samples were extracted, cloned, purified, sequenced and are still being evaluated by our research team. A few bands returned successful sequencing results and are detailed in Table 3.
Table 3: Sample Sequence Information. The following table gives the conditions of sequenced samples following cloning and extraction of plasmid DNA.

<table>
<thead>
<tr>
<th>Sequence ID</th>
<th>Cat #</th>
<th>Sample #</th>
<th>Tissue Type</th>
<th>Restriction Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>2338683</td>
<td>23</td>
<td>6</td>
<td>Brain</td>
<td>PvuII-HF</td>
</tr>
<tr>
<td>2338684</td>
<td>23</td>
<td>6</td>
<td>Brain</td>
<td>Dral</td>
</tr>
<tr>
<td>2338679</td>
<td>23</td>
<td>2</td>
<td>Lymph</td>
<td>Dral</td>
</tr>
<tr>
<td>2338669</td>
<td>23</td>
<td>4</td>
<td>Lymph</td>
<td>Dral</td>
</tr>
<tr>
<td>2338680</td>
<td>23</td>
<td>3</td>
<td>Lymph</td>
<td>Dral</td>
</tr>
<tr>
<td>2338682</td>
<td>23</td>
<td>5</td>
<td>Brain</td>
<td>PvuII-HF</td>
</tr>
<tr>
<td>2338678</td>
<td>23</td>
<td>1</td>
<td>Lymph</td>
<td>Dral</td>
</tr>
</tbody>
</table>

Upon receiving sequencing data from these samples, the Clustal bioinformatics computer program was utilized for multiple sequence alignment. The alignments of these sequences are shown in Figure 11.
Figure 11: Clustal Multiple Sequence Alignment. This figure details the alignment of sequences from brain and lymph samples. Sequences are continued in seven consecutive rows with sample IDs listed on left. The only mismatches seen in the alignment are at the beginning and end of the sequences, where the base pair assignments are less reliable.
The sequences shown in Figure 11 align to one another with no mismatches which provides confidence in our methodology and sequence results. The presence of bands in both primary and secondary PCR and data returned from sequencing demonstrates successful amplification of exogenous FeLV-B sequences from brain tissue, indicating that feline brain tissue has cellular receptors which are recognized by FeLV-B. This is significant because previous research with FeLV-C suggested that brain cells have a resistance to the virus and that only endogenous FeLV-B sequences would be found within the central nervous system.\textsuperscript{15}

Furthermore, the research performed was successful in designing primers. Primers were designed based on sequencing results and are in use with optimized PCR conditions allowing our research team to move forward and sequence the entire proviral insert and adjacent host genes.

**Future Directions**

Future directions include continued sequencing of the entire proviral insert and adjacent host genes to help better understand how insertion sites relate with disease manifestation and progression. Primer walking using optimized methodology is being performed to determine proviral sequences in tissue samples, as well as the insertion site of the provirus. Results of tissue samples will be compared to those of blood samples to note any differences between the sequences. Furthermore, we plan to translate sequences to the protein it would produce in order to detail differences in our sequences to mutations that have been characterized in literature.
The FeLV genome we sequenced is similar to sequences of samples collected worldwide, with the exception some differences in a small region of the LTR. Natural isolates of virus from small geographical regions have unique sequences in the LTR, which are thought to lead to varied disease progression. Further research will yield detailed sequence information for a close group of cats in southeast Michigan that can be compared to the LTR for other natural strains of FeLV, and correlated with disease progression within the group.

References


Chakrabarti, R.; Hofman, F. M.; Pandey, R.; Mathes, L. E.; Roy-Burman, P.

Abstract

Feline Leukemia Virus (FeLV) is a transmittable RNA retrovirus that inhibits feline immune systems and predisposes its host to infections and diseases. Much is unknown about FeLV, including the nature and location of its insertion sites. Identifying insertion sites of the provirus can provide information about mechanisms of tumorigenesis and identify new proto-oncogenes. To search for insertion sites, gene-specific primers were designed and a genome walking method was optimized for our application. Using the optimized process, blood and tissue samples were examined for FeLV provirus. The cat from which we received samples showed neurological symptoms before death; therefore, we hypothesized FeLV sequences would be present in brain tissue. We also hypothesized that brain sequences and insertion sites would differ from specific sequences found in blood and other tissue samples. Exogenous FeLV-B sequences were successfully amplified from blood, brain and lymph tissues and no differences were found between sequences. Exogenous sequences in the brain indicate the presence of cellular receptors recognizable by FeLV-B. This is significant because previous research suggested that brain cells have a resistance to the virus. Furthering knowledge of mechanisms and locations of insertion can lead to a more complete understanding of FeLV disease progression, ultimately leading to the development of more effective treatments, conserving the health of domesticated and large endangered feline species.